TECHNICAL MANUAL

Pyruvate Kinase (PK) Activity Assay Kit

- **SKU CODE:** MAES0247
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Sample preparation
- Reagent preparation
- Add sample and reagents to microplate
- Measure absorbance at 20 seconds and 3 minutes 20 seconds
- Calculate PK activity

2. Intended use

This kit can be used to measure pyruvate kinase (PK) activity in serum, plasma, tissue and cell samples.

3. Detection principle

In the presence of adenosine diphosphate (ADP), pyruvate kinase (PK) catalyzes the conversion of phosphoenolpyruvate (PEP) to pyruvate. Lactate dehydrogenase catalyzes the reaction of pyruvic acid with NADH to produce lactic acid and NAD+. The activity of pyruvate kinase can be calculated by detecting the absorbance changes at 340 nm.

4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	10 mL x 1 vial	10 mL x 2 vials	-20 °C, 12 months
Reagent 2	Substrate A	Powder x 1 vial	Powder x 2 vials	-20 °C, 12 months, shading light
Reagent 3	Substrate B	Powder x 1 vial	Powder x 2 vials	-20 °C, 12 months, shading light
Reagent 4	Enzyme Solution	1.2 mL x 1 vial	1.2 mL x 2 vials	-20 °C, 12 months, shading light
	UV Microplate	96 wells	96 wells	No requirement
	Plate Sealer	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

Micropipettor, Vortex mixer, Incubator, Microplate reader (330-350 nm, optimum wavelength: 340 nm)

Reagents:

Normal saline (0.9% NaCl)

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of substrate A working solution:** Dissolve one vial of substrate A with 1.2 mL of double distilled water, mix well to dissolve. Store at -20 °C for 3 days protected from light.
3. **Preparation of substrate B working solution:** Dissolve one vial of substrate B with 10 mL of buffer solution, mix well to dissolve. Store at -20 °C for 3 days protected from light.
4. **Preparation of enzyme working solution:** For each well, prepare 40 µL of enzyme working solution (mix well 20 µL of substrate A working solution and 20 µL of enzyme solution). The enzyme working solution should be prepared fresh and used up on the same day.

7. Sample preparation

Sample preparation:

Serum and plasma: Detect directly. If not detected on the same day, the serum or plasma can be stored at -80 °C for a month.

Tissue sample:

1. Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

2. Wash tissue in cold PBS (0.01 M, pH 7.4).

3. Homogenize 20 mg tissue in 180 µL normal saline (0.9% NaCl) with a dounce homogenizer at 4 °C.

4. Centrifuge at 10,000 x g for 10 minutes at 4 °C to remove insoluble material. Collect supernatant and keep it on ice for detection.

5. Meanwhile, determine the protein concentration of supernatant (MAES0177).

Cell (adherent or suspension) samples:

1. Harvest the number of cells needed for each assay (initial recommendation 1 x 10^6 cells).

2. Wash cells with PBS (0.01 M, pH 7.4).

3. Homogenize 1 x 10^6 cells in 200 µL normal saline (0.9% NaCl) with an ultrasonic cell disruptor at 4 °C.

4. Centrifuge at 10,000 x g for 10 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection.

5. Meanwhile, determine the protein concentration of supernatant (MAES0177).

Dilution of sample:

The recommended dilution factor for different samples is as follows (for reference only):

- 10% Rat kidney tissue homogenate: 4-6
- 10% Rat lung tissue homogenate: 4-6
- 10% Rat liver tissue homogenate: 4-6
- 10% Rat brain tissue homogenate: 4-6
- 10% Mouse liver tissue homogenate: 4-6
- 10% Mouse heart tissue homogenate: 4-6
- Rat serum: 1
- Rat plasma: 1
- Mouse serum: 1
- Mouse plasma: 1
- Human serum: 1
- Dog plasma: 1
- HL-60 cell: 1
- 293T cell: 1

Note: The diluent is normal saline (0.9% NaCl). For the dilution of other sample types, please perform a pretest to confirm the dilution factor.

8. The key points of the assay

1. The sample for detection should be as fresh as possible, as the activity of PK will be reduced during long-term storage.
2. The time of A2 can be extended if the value of serum sample is too low.

9. Operating steps

1. Sample well: Add 10 µL of sample to the wells.

2. Add 150 µL of substrate B working solution and 40 µL of enzyme working solution into each well.
3. Measure the OD value of each well at 20 s and 3 min 20 s respectively at 340 nm with microplate reader, recorded as A1, A2, ΔA = A1 - A2.

10. Calculation

The sample:

1. Serum (plasma) sample:

Unit definition: the enzyme amount of 1 mmol of NADH consumed by 1 L of liquid sample per minute at room temperature is defined as 1 unit

PK activity (U/L) = (ΔA340 / (6220 × d × V_{Total})) ÷ V_{Sample} ÷ T × f × 1000

2. Tissue and cells samples:

Unit definition: the enzyme amount of 1 mmol of NADH consumed by 1 g sample protein per minute at room temperature is defined as 1 unit

PK activity (U/gprot) = (ΔA340 / (6220 × d × V_{Total})) ÷ (V_{Sample} × C_{pr}) ÷ T × f × 1000

[Note]

ΔA340: A_2 - A_1.

6220: The molar extinction coefficient of NADH, L/mol·cm

d: Optical path, 0.6 cm.

V_{Total}: The total volume of the reaction system, 0.2 mL.

V_{Sample}: The volume of the sample, 0.01 mL.

C_{pr}: Concentration of protein in sample, gprot/L.

T: The time of reaction, 3 min.

f: Dilution factor of sample before test.

1000: 1 mol/L = 1000 mmol/L.

11. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Intra-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	1.20	3.40	5.80
%CV	5.3	5.0	4.7

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	1.20	3.40	5.80
%CV	6.8	7.3	6.9

Recovery

Three samples of high concentration, middle concentration and low concentration were tested with each concentration measured 6 times in parallel to obtain an average recovery rate of 103%.

Recovery

Parameters	Sample 1	Sample 2	Sample 3
Expected Conc. (U/L)	1.8	3.7	6.2
Observed Conc. (U/L)	1.8	3.9	6.5
Recovery rate (%)	99	105	105

Sensitivity

The analytical sensitivity of the assay is 0.08 U/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

12. Appendix II Example Analysis

Example analysis:

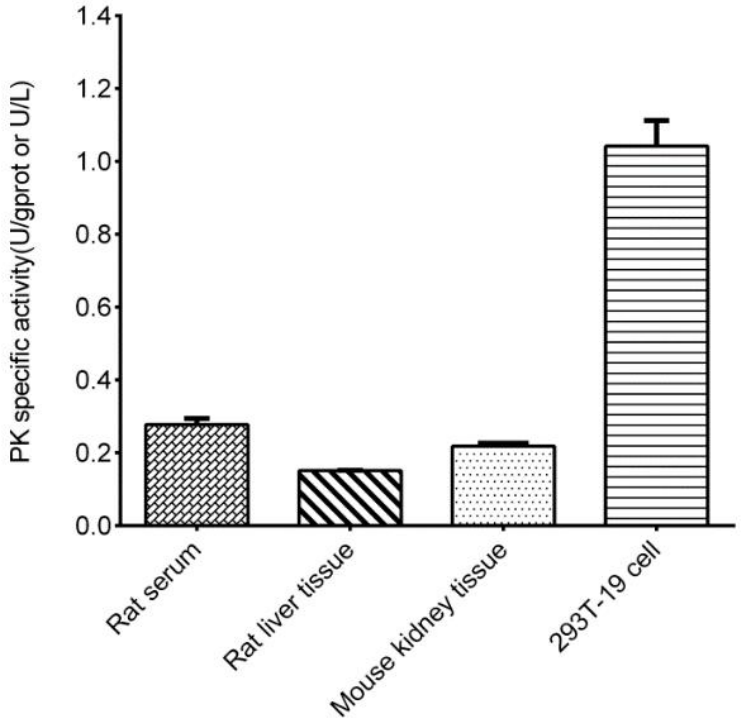
For rat liver tissue, take 10 μL of 10% rat liver tissue homogenate, dilute 5 times, and carry out the assay according to the operation steps. The results are as follows:

The A_1 of the sample is 1.043, after 3 minutes of reaction, the A_2 of the sample is 0.892, the concentration of protein in sample is 8.95 gprot/L, and the calculation result is:

PK activity (U/gprot) = (1.043 - 0.892) ÷ (6220 × 0.6) × 0.2 ÷ (0.01 × 8.95) ÷ 3 × 5 × 1000

= 0.15 U/gprot

Detect rat serum, 10% rat liver tissue homogenate (the concentration of protein is 8.95 gprot/L, diluted 5 times), 10% mouse kidney tissue homogenate (the concentration of protein is 9.52 gprot/L, diluted 5 times) and 293T cell (the concentration of protein is 1.80 gprot/L) according to the protocol, the result is as follows:



13. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.
2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.
3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.
4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.
5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.
6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

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Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.